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Distribution of Carbohydrates during a Diatom Bloom in the Northern Adriatic*

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Distribution of carbohydrates was studied in the northern Adriatic during an autumn phytoplankton bloom triggered by strong freshwater discharges from the Po River at the end of the stratification period in October 1993. Total carbohydrates (TCHO) and monosaccharides (MCHO) were determined spectrophotometrically using the MBTH-method, while phytoplankton biomass was followed by determining chlorophyll and carotenoid pigments using the HPLC technique. As indicated by the concomitant increase of chlorophyll *a* and fucoxanthin, an intensive diatom bloom developed in the upper 5 m of the water column while the chlorophyll biomass was rather low in the layers below 10 m. Total carbohydrate levels varied from 70 μg C/l to 1300 μg C/l with significantly enhanced values in the top 5 m of the water column suggesting a strong link to the diatom biomass. However, it was shown that not only the diatom crop but also the physiological status of the biomass, as reflected by the chlorophyll break-down products, had a strong impact on the TCHO levels. Carbohydrates were found mainly in the form of polysaccharides (up to 92% of TCHO).

INTRODUCTION

Carbohydrates (CHO) are one of the largest fractions of the dissolved organic carbon (DOC) in the marine environment. Various earlier studies showed that carbohydrates comprised about 15% of DOC, while a recent report by Pakulski and Benner[1] on the carbohydrate distribution in surface

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waters of the world oceans gave an even higher estimate of 21 ± 7%. Due to the enhanced biological activity in neritic areas, the importance of carbohydrates in estuarine and coastal waters could be even greater; however, there are only a few reports dealing with such ecosystems.[2,3] Senior and Chevolot[3] reported on large temporal and spatial variations of carbohydrates along the salinity gradients of a small estuary (Elorn, France) with maximum concentrations (up to 1080 μg C/l) in June, which was attributed to the phytoplankton-derived polysaccharides. In other seasons, most carbohydrates were low and fairly well correlated with DOC, which suggested their link to a conservative fraction of organic matter. Investigations of the concentration and chemical nature of polysaccharides conducted in Mikawa Bay (Japan) during a red tide bloom of *Prorocentum minimum* also pointed to the important role of phytoplankton extracellular release and/or cell lysis in the production of dissolved polysaccharides in marine water.[4] Studies performed during a diatom bloom in the North Sea[5,6] showed that large amounts of carbohydrates were released into seawater towards the end of the bloom, with a considerable percentage being in the combined form. Diatom blooms are very common for coastal areas and they play a major role in the phytoplankton dynamics and other eutrophication-related processes in the northern Adriatic.[7]

It was suggested that diatoms could have been an important source of carbohydrates that eventually led to hypertrophic formation of gelatinous aggregates observed in the northern Adriatic.[8] However, very little is known about the origin, occurrence and distribution of carbohydrates in the northern Adriatic, especially about their relationship with phytoplankton.[9] A weak but statistically significant correlation between the number of diatom cells and the concentration of total carbohydrates was noticed in a preliminary study conducted in the northern Adriatic in 1992.[10] Moreover, seasonal variation of particulate carbohydrates in the Gulf of Trieste was studied by Faganeli *et al.*,[11] who found a good correlation between particulate carbohydrates and phytoplankton biomass. Enhanced concentrations of particulate carbohydrates (> 100 μg/l) were found in the period characterized by macroaggregate formation while in normal situations their contribution to the total particulate organic carbon was below 10%.

The aim of this paper was to determine the impact of a major diatom bloom that occurred in the northern Adriatic in October 1993 on the concentration levels and spatial distribution of carbohydrates.

EXPERIMENTAL

Study Area and Sampling Strategy

The northern Adriatic is a shallow semi-enclosed basin (maximum depth 35 m) that receives large freshwater inputs from the Po River and other North Italian riv-

ers. These discharges are responsible for the marked gradients in physical, chemical and biological properties, including eutrophication, present along the transects from the western to the eastern coast.[12] In a typical winter situation, the predominant circulation pattern in the northern Adriatic leads to export of highly eutrophic waters along the Italian coast so that the basin interior remains hardly affected (see Ref. 13 for review). In contrast, during the period of summer stratification, the circulation pattern is usually altered by formation of a cyclonic gyre which prevents efficient export of water from the region and supports the spreading of freshwater inputs into the inner parts of the basin. Such a hydrodynamic feature was shown to be responsi-

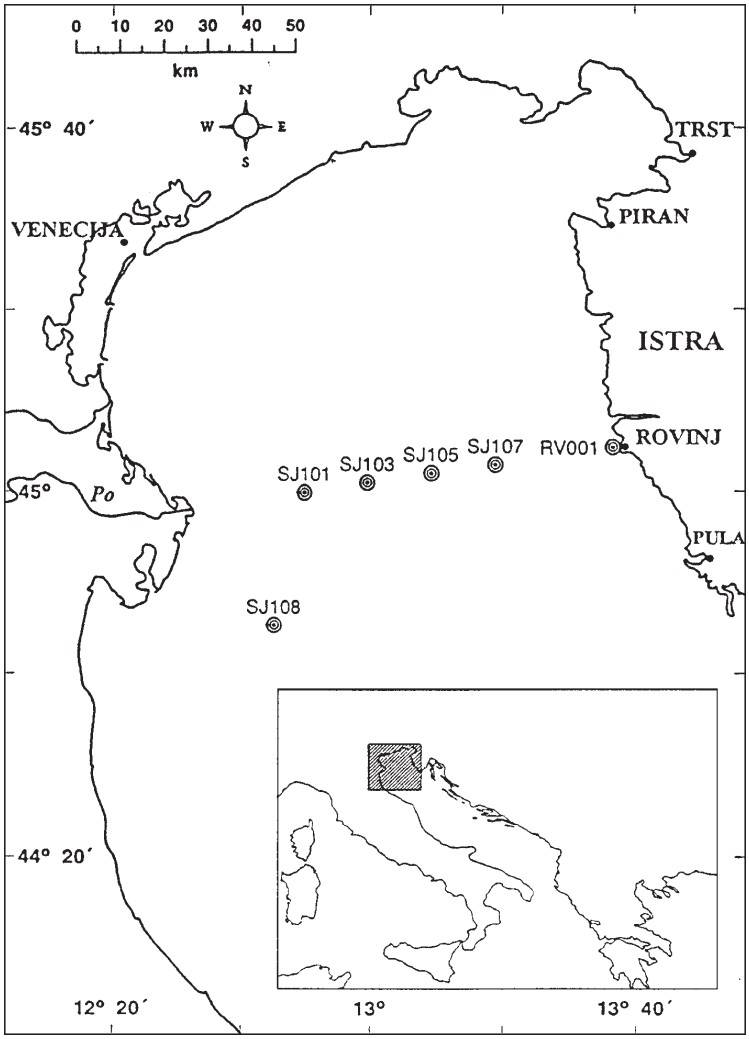

Figure 1. Map of the northern Adriatic with sampling stations on the transect: Po River mouth – Rovinj.

ble for comparatively enhanced eutrophication of the eastern side of the northern Adriatic during summer stratification.[14] The Po River discharges are commonly low during summer, while the maxima are regularly observed in spring and autumn. Recent analyses of long term trends in the seasonal pattern of the Po River discharges[15] showed, however, that in the last two decades earlier occurrences of the autumn maximum, in October instead of November, were more frequent. The main difference between the two timings is that the water column in November becomes well-mixed while it is usually still stratified in October, allowing a more efficient lateral advection of freshwaters towards the eastern side of the basin.[15] Such a situation occurred in October 1993, when very high Po River discharges flooded the whole northern Adriatic, leading to a significantly decreased salinity and high nutrient levels in the top 5 m of the water column.[16]

Samples for the carbohydrate and pigment analyses were collected in the central part of the northern Adriatic on the transect Po River mouth-Rovinj (Figure 1). The stations along this transect are generally accepted as representative of eutrophication gradients in the northern Adriatic, especially during summer stratification.[14,15] Sampling was performed on 18 October 1993 from the research vessel »Vila Velebita« at 6 stations and at 5–6 depths (0, 5, 10, 20, 30 m and near the bottom) using 5 l Niskin bottles. Unfiltered seawater samples were frozen immediatelly after sampling and were kept at –20 °C until analyzed. The samples for pigment analyses were filtered on board and immediatelly frozen at –20 °C.

Determination of Carbohydrates

Dissolved and particulate monosaccharides as well total carbohydrates were determined in nonfiltered seawater samples using the MBTH method. Determination of monosaccharides followed the original procedure of Johnson and Sieburth,[17] while total carbohydrates[18] were determined by HCl hydrolysis.[3] Briefly, monosaccharides were reduced to the corresponding alditols with sodium borohydride. The alditols were subsequently oxidized with periodic acid to yield 2 mol of formaldehyde per mol of monosaccharide. Formaldehyde concentration was finally determined spectrophotometrically after the reaction with MBTH. In control samples (blanks), which were analyzed daily along with each series of samples, the oxidation of alditols was prevented by the premixing of periodic acid and sodium arsenite solutions before their addition to the sample.

The hydrolysis was performed using a method modified from Senior and Chevolot.[3] Five-ml samples were placed in glass test tubes (13 × 100 mm) equipped with Teflon-lined caps and acidified with 1 ml of 30% hydrochloric acid. Samples were hydrolyzed in a water bath at 100 °C for 3.5 h. The cooled hydrolysate was neutralised with 1.1 ml of 10 mol/l NaOH.

Samples were analyzed in triplicate and quantification was performed using a calibration curve for glucose standard solutions in a range from 0.5 to 15 μmol/l. Sample absorbances of hydrolyzed samples were multiplied by 1.3 to compensate for sample dilution during the hydrolysis and neutralization. All concentrations are expressed in glucose carbon equivalents by multiplying the weight glucose equivalents by 0.4, since glucose is 40% carbon by weight. The reproducibility of the carbohydrate determination was < 5% for the higher concentration range (> 200 μg C/l) and < 12% for the lowest concentrations. The limit of detection was 30 μg C/l.

Determination of Biomarker Pigments and Breakdown Products of Chlorophyll a

Samples for the photosynthetic pigment analyses (0.5 l) were filtered onto 25 mm GF/F filters. Filters were immediately stored at –20 °C until analysis. The filters were extracted in 3 ml of cold 90% acetone using sonication, centrifuged to clarify the extract, and the chlorophylls and carotenoids separated by reversed-phase HPLC according to Barlow *et al.*[19] Briefly, extracts were mixed (1:1; v/v) with 1 M ammonium acetate and injected into a HPLC system consisting of a gradient solvent delivery system (Varian Star 9010), injector (Rheodyne, Model 7125), C_{18} 3 μm Pecosphere column (3.3 × 0.45 cm, Perkin Elmer) and serially coupled spectrophotometric and spectrofluorimetric detectors. A binary linear gradient was used to separate the pigments. Solvent A consisted of methanol (ϕ = 0.80) and 1 M ammonium acetate (ϕ = 0.20), while solvent B contained methanol (ϕ = 0.60) and acetone (ϕ = 0.40). Chlorophylls and carotenoids were detected by absorbance at 440 nm (Spectra Physics UV 2000), while phaeopigments were detected by fluorescence (Spectra Physics F 2000) using excitation at 420 nm and emission at 672 nm. Data collection and reprocessing utilized the Spectra Physics PC 1000 software. Qualitative identification and quantitative determination of individual pigments was performed according to Barlow *et al.*[19] and Terzić.[9] The reproducibility of pigment determination was around 5%, while the detection limits varied from 1–5 ng/l, depending on the pigment extinction coefficients at 440 nm.

RESULTS

Distributions of salinity, nitrate and orthosilicate in the upper part of the water column (layers at 0, 5 and 10 m) of the northern Adriatic show that the top five meters were strongly influenced by freshwater discharges from the Po River along the entire transect SJ108–RV001 (Figure 2). Salinities in the surface layer (0 m) varied from 21.89 to 31.59. In fact, apart from the significantly lower salinity at station SJ108 (21.89), there was only a slight gradient on the transect between stations SJ101 and RV001 (salinities between 28.98–31.59). Such distribution of surface salinities was reflected in the distribution of nutrients. The two most abundant riverborne nutrients, nitrate and orthosilicate, were present in the surface layer at station SJ108 in high concentrations of 20.5 μmol/l and 39.4 μmol/l, respectively. The concentration of nitrate on the transect from SJ101 to SJ107 was significantly lower but rather constant (from 6.2 to 7.1 μmol/l), while orthosilicate concentration decreased from 3.5 μmol/l at station SJ101 to 1.3 μmol/l at station SJ107. Both nutrients showed a non-conservative behaviour, departing from the expected dilution curve. In the subsurface layer (5 m), both nutrients were severely depleted by comparison with the surface layer. Except at station SJ108, both nitrate and orthosilicate were below or around 2 μmol/l. It is interesting to stress that vertical distribution of nutrients showed that the freshwater input had no impact on their concentration in the intermediate layer (20 m). In addition, intensive regeneration processes

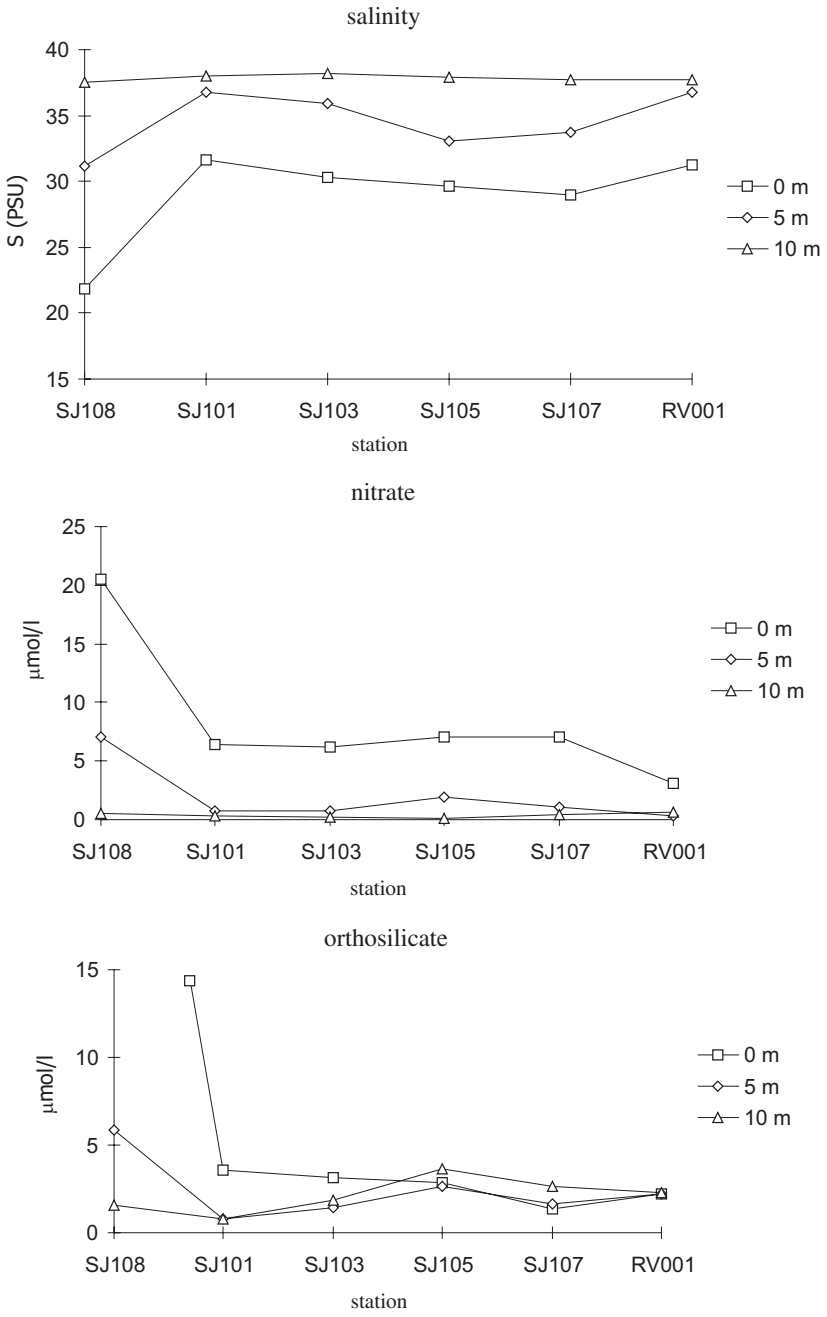

Figure 2. Salinity and nutrient distribution in 3 different layers of the top 10 meters along the transect: Po River mouth – Rovinj in October 1993 (data courtesy of the Center for Marine Research, Rovinj, Croatia).

in the bottom layer, as indicated by enhanced levels of orthosilicate (3.8––18.5 μmol/l), ammonia (1.1–3.9 μmol/l) and orthophosphate (0.03–0.58 μmol/l) as well as by lower oxygen saturation percentages[16] (not presented in Figure 2), were efficiently decoupled from the biological processes in the upper layers by a well-stratified water column.

Nutrient distribution described above determined the spatial distribution of the phytoplankton biomass in the basin. The highest chlorophyll *a* concentration levels (Figure 3) were found in the top 5 m of the water column (from 3000 ng/l at station RV001 up to 6100 ng/l at station SJ101). The concentration of chlorophyll *a* at 10 m was much lower (460–2000 ng/l) but still

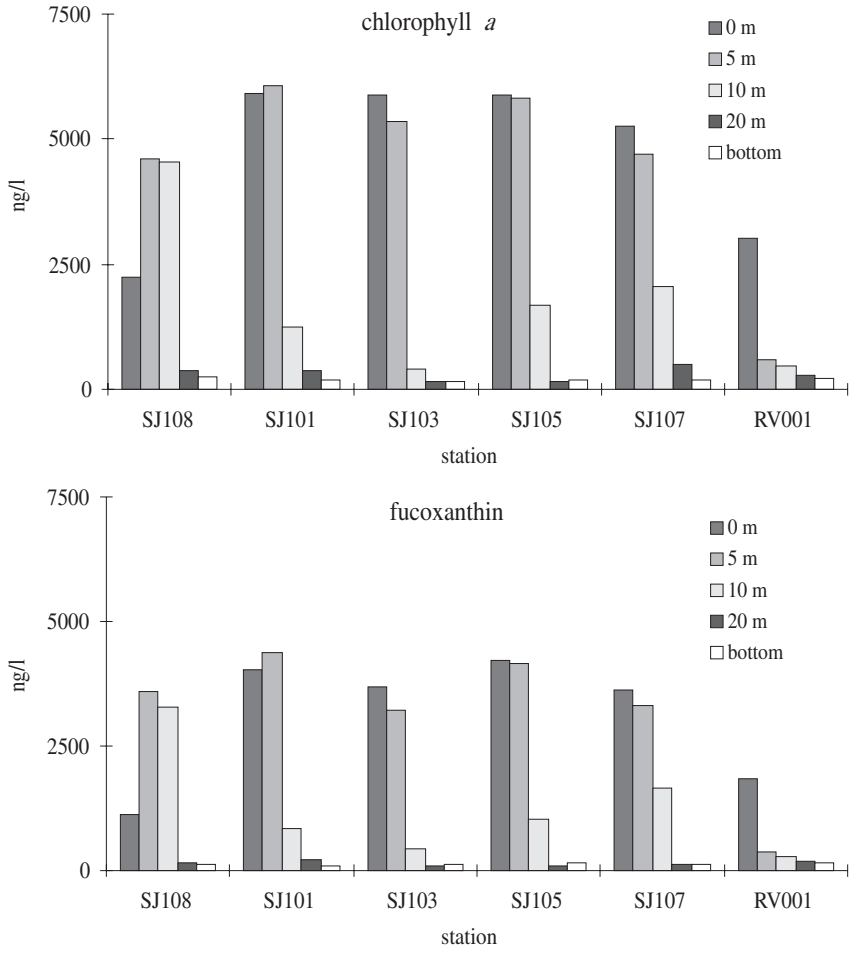

Figure 3. Spatial and vertical distribution of chlorophyll *a* and fucoxanthin at 6 stations on the transect: Po River mouth – Rovinj in October 1993.

enhanced, reflecting the influence of the overlying layers. An exception was station SJ108, which was under the strongest impact of freshwater discharges, showing a comparatively lower concentration at the surface (2300 ng/l) than at 10 m (4600 ng/l). The layers below 10 m, including the bottom layer which was rich in all nutrients, were characterized by very low chlorophyll *a* concentrations, ranging from 120 to 500 ng/l. The spatial distribution of chlorophyll *a* in the surface layer along the transect SJ101–RV001 is in very good agreement with the nutrient distribution. High and rather uniform concentrations were found in the top 5 m in the middle part of the transect (stations SJ101 to SJ107; 4700–6100 ng/l), while the concentration at station RV001 was significantly lower (600–3000 ng/l), with a pronounced difference between the surface (0 m) and subsurface (5 m) layers. The spatial distribution of chlorophyll *a* in the bottom layer had no similarity to that in the surface layer, which indicated an efficient decoupling of the two layers.

Composition of biomarker pigments was relatively simple, both in the surface and bottom layers (Figure 4). Fucoxanthin was the most prominent accessory pigment in both layers (50–96% of the total biomarker pigments),

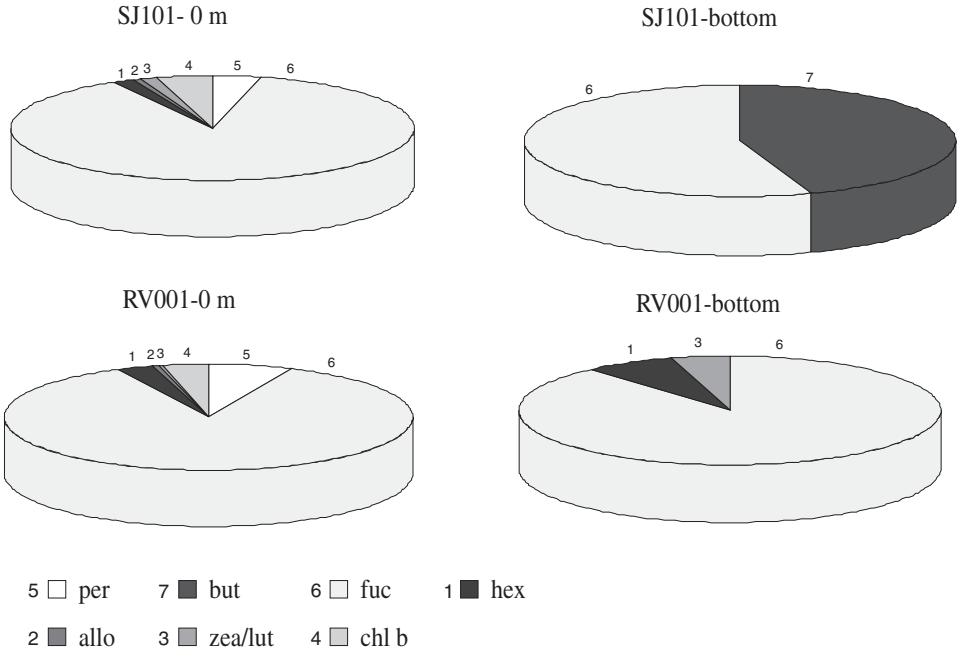

Figure 4. Composition of biomarker pigments in the surface and bottom layers of two selected stations situated in the western (SJ101) and eastern (RV001) parts of the transect: Po River mouth – Rovinj in October 1993. Pigment identities are per: peridinin; but: 19′-butanoyloxyfucoxanthin; fuc: fucoxanthin; hex: 19′-hexanoyloxyfucoxanthin; allo: alloxanthin; zea/lut: zeaxanthin/lutein; chl *b*: chlorophyll *b*.

pointing to the predominance of diatoms. Minor biomarker pigments detected in analyzed samples were peridinin, chlorophyll *b*, 19′-hexanoyloxyfucoxanthin, 19′-butanoyloxyfucoxanthin, zeaxanthin and alloxanthin, indicating the presence of dinoflagellates, green algae, prymnesiophytes, chrysophytes, cyanobacteria and criptophytes, respectively.[20,21] The phytoplankton composition, as reflected in biomarker pigments, was more complex in the surface layer: however, no major differences were observed between the western (SJ101) and eastern (RV001) sides of the transect. In contrast, compositions of biomarker pigments in the bottom layers of the two stations were rather different. High contribution (45%) of 19′-butanoyloxyfucoxanthin at stations SJ101 (Figure 4) and SJ108 indicated a significant presence of chrysophytes (silicoflagellates) in the bottom layer of the western part of the basin, very probably in response to increased availability of regenerated biogenic orthosilicate (15.9–18.5 μmol/l). 19′-butanoyloxyfucoxanthin was not detected in the bottom layer of the eastern part of the basin (RV001), which showed a very strong predominance of diatoms (fucoxanthin), with minor contribution of prymnesiophytes (19′-hexanoyloxyfucoxanthin) and cyanobacteria (zeaxanthin).

Since fucoxanthin was such a dominant biomarker pigment its spatial distribution along the transect as well as on the vertical profile (Figure 3b) closely matched the pattern of chlorophyll *a* (correlation coefficient $r^2 = 0.98$). Concentrations in the brackish and bottom layers varied from 1100 to 4400 ng/l and from 60–200 ng/l, respectively. The chlorophyll-to-fucoxanthin ratios were 1.4–2.0 in the surface layer and 1.3–2.0 in the bottom layer. No significant difference was observed between the two ratios despite the fact that the two populations were separated by a large intermediate layer of the well-stratified water column and were exposed to different light conditions.

Four major breakdown products were detected in the samples: chlorophyllide *a*, phaeophorbide a_1, phaeophorbide a_2 (pyrophaeophorbide) and phaeophytin a_1. In contrast to the relatively simple distribution of taxonomic pigments, the composition and pattern of chlorophyll *a* breakdown products was very complex (Figure 5). The most abundant individual breakdown product was chlorophyllide *a* with concentrations reaching up to 300 ng/l, while all other breakdown products remained below 100 ng/l. The spatial distribution of chlorophyllide *a* showed a maximum in the surface layer of the eastern part of the basin (stations SJ107 and RV001). There was no significant correlation of phaeopigments with chlorophyll *a* in that layer, which contained an enhanced phytoplankton biomass (chlorophyll *a*). Concentrations of chlorophyllide *a* in the layers below 5 m were around the detection limit (1–10 ng/l). It is also interesting to note that chlorophyllide *a* was not detectable at the westernmost station SJ108. A very similar pattern to that one of chlorophyllide *a* was observed for phaeophytin a_1, but in a lower concentration range (1–60 ng/l). The other two breakdown products, phaeophorbide a_1 and phaeophorbide a_2, showed much more complex distributions

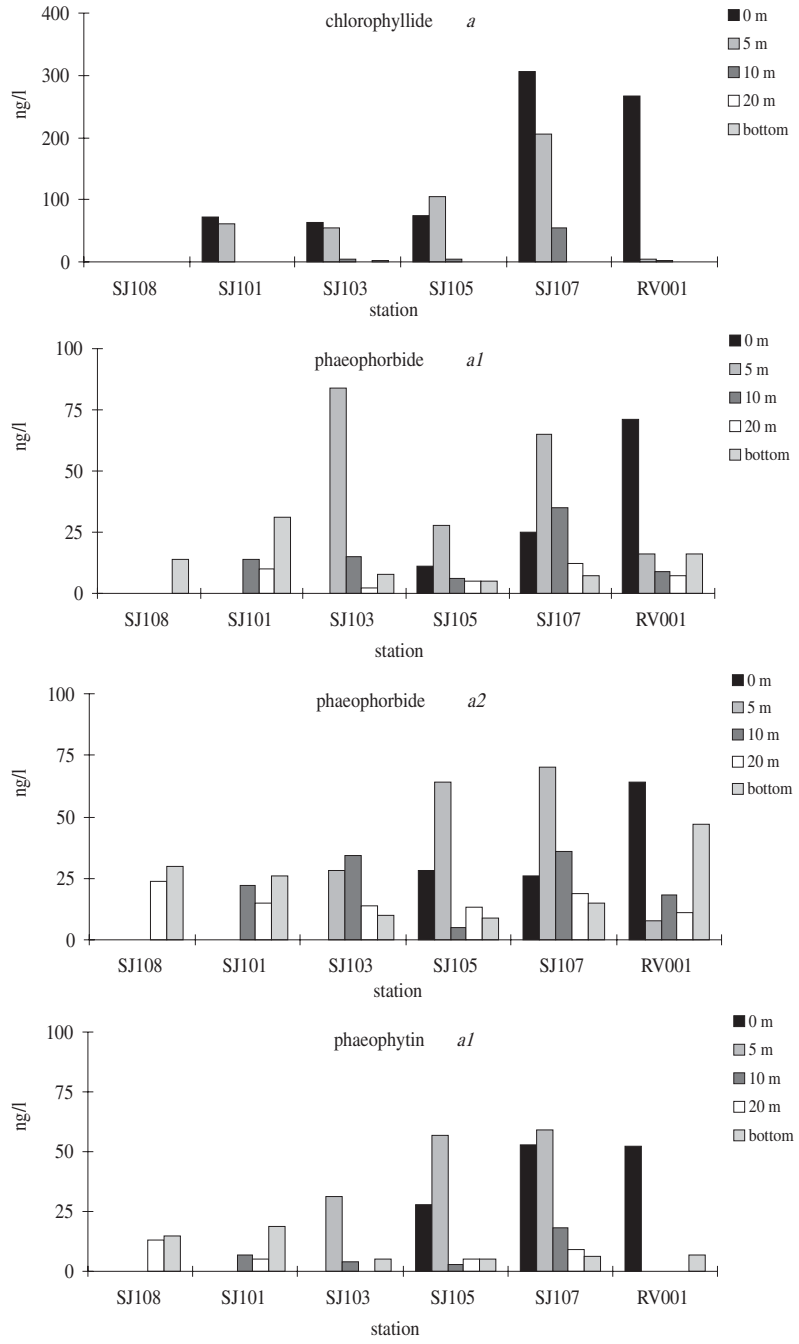

Figure 5. Spatial and vertical distribution of 4 selected breakdown products of chlorophyll *a* on the transect: Po River mouth – Rovinj in October 1993.

with a great deal of mutual similarities. The only significant difference was an enhanced concentration of phaeophorbide a_1 in the surface layer of station SJ103. Otherwise, the maxima of both phaeophorbides were found in the 5 m-layer at station SJ107. In contrast to the chlorophyllide *a* pattern, low (1–30 ng/l) but significant concentrations of phaeophorbide a_1 and phaeophorbide a_2 were found in deeper layers, including station SJ108. The total concentration of phaeopigments (obtained as a sum of individual compounds discussed above) accounted for < 1–15% (mass fraction) of chlorophyll *a* in the surface layer, with the highest value in the eastern part of the basin. In the bottom layer, however, the percentage was significantly higher (10–44%), and the highest value were found in the western part of the basin (station SJ101).

Analyses of carbohydrates included determination of both the free (before hydrolysis) and total carbohydrates (after hydrolysis), while combined carbohydrates (polysaccharides) were determined as their difference. It should be pointed out that only unfiltered samples were analyzed, so the obtained results represent the total concentration (dissolved + particulate). Furthermore, the hydrolysis for TCHO determination was performed with hydrochloric acid. It was shown by Pakulski and Benner[22] that estimates of carbohydrates in natural samples after hydrolysis with dilute HCl (0.1 mol/l) were typically half those obtained by sulphuric acid hydrolysis since structural polysaccharides, such as cellulose and chitin, cannot be efficiently hydrolyzed by dilute HCl. However, in our case, the majority of carbohydrates originated from phytoplankton and the hydrolysis was performed with a much stronger HCl solution (1.7 mol/l). We assume therefore that the determined carbohydrate concentrations should be very close to true values. The concentration of total carbohydrates varied in a very wide range from 70 μg C/l to 1300 μg C/l, while the concentration of monosaccharides was much lower (20–240 μg C/l). The distribution of various forms of carbohydrates presented in Figure 6 showed an interesting pattern, which was markedly different from that for chlorophyll *a* (Figure 3). Significantly higher concentrations of total carbohydrates were found in the central and eastern part of the basin (stations SJ105, SJ107 and RV001) than in the western part (SJ108, SJ101, SJ103). Moreover, a very pronounced difference of concentration levels in the top 5 m layer (330–1300 μg C/l) and those in deeper layers (70–230 μg C/l) was recorded. The only exception was station SJ108 with the highest total carbohydrate concentration in the layer at 10 m (690 μg C/l), while at all the other stations the total carbohydrate concentration never exceeded 300 μg C/l in layers bellow 5 m. The most interesting results are the extremely high concentrations of total carbohydrates in the surface layer of stations SJ105, SJ107 and RV001 (900–1300 μg C/l). Most of the detected carbohydrates were in the form of polysaccharides, which consequently showed a distribution pattern almost identical to total carbohydrates. Maximum concentrations of polysaccharides were determined in the

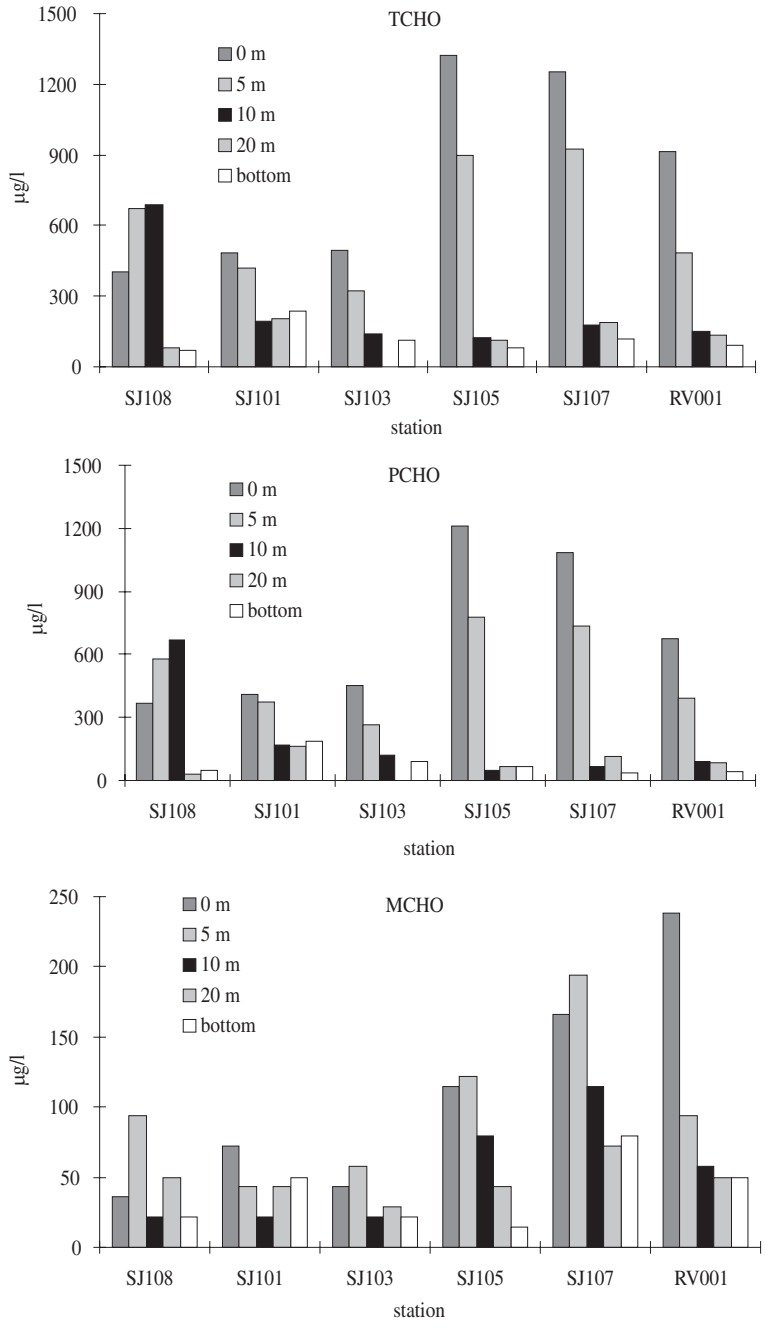

Figure 6. Spatial and vertical distribution of total carbohydrates (TCHO), polysaccharides (PCHO) and monosaccharides (MCHO) on the transect: Po River mouth – Rovinj in October 1993.

surface layer of the central and eastern parts of the basin (680–1200 μg C/l), representing a very high percentage of total carbohydrates (up to 92%). The percentage of polysaccharides was lower in deeper layers (below 5 m), however, still being dominanted by the carbohydrate form. Although monosaccharides represented a minor fraction of total carbohydrates, their distribution pattern along the transect SJ101–RV001 is very interesting. The concentration of monosaccharides was not well correlated with the concentration of total carbohydrates but it showed a continuous increase towards the eastern part of the basin with the highest concentration determined in the surface layer at station RV001 (240 μg C/l). The most frequent concentration values of monosaccharides were well below 100 μg C/l. Absolute values of monosaccharides were greater in the surface (0 m) and subsurface (5 m) layers (40–240 μg C/l) than in the lower layers (10–80 μg C/l), however, the percentage of monosaccharides in total carbohydrates increased with depth. Consequently, the vertical gradient of monosaccharides in the water column was not as sharp as for total carbohydrates. It is interesting to point to the similarity between the distribution patterns of total carbohydrates (Figure 6) and chlorophyllide *a* (Figure 5).

DISCUSSION

Rather unusual heavy rains in the Po River watershed and subsequent continuing high freshwater discharges into the northern Adriatic during the early autumn of 1993 created a unique hydrological situation in the basin, which had an immense impact on the biology and chemistry of the whole basin. Since the water column was still well stratified, the spreading of freshwater into the basin interior was very efficient. In fact, cooler (< 19 °C) river water was sliding very fast over the warmer (> 20 °C) seawater column and eventually reached as far as the coastal waters of western Istria (Figure 2). Such an enhanced lateral advection of freshwaters was shown to be characteristic of the summer season when typical cyclonic current system along the western coast of the northern Adriatic is diverted,[13] which allows spreading of freshwaters into the basin interior. This mechanism is responsible for the enhanced lateral transport of particulate organic carbon, notably phytoplankton, and it is largely influenced by the hydrodynamic regime of the Po River.[14,15]

Entrainment of the underlying seawater during the flooding of the northern Adriatic in October 1993 resulted in formation of a 5–10 m thick brackish layer across the whole basin which was well-separated from deeper layers. Such hydrographic properties of the basin had a profound effect on the distribution of phytoplankton. In response to nutrient-rich freshwater inputs, a strong diatom bloom developed, but was confined only to the brackish layer, while the phytoplankton crop in the underlying layers was

very low, which is characteristic of the stratification period.[23,24] It is interesting to note that the chlorophyll *a* concentration in the brackish layer was more than 20 times higher than in the bottom layer, although there was no nutrient limitation in the bottom layer due to the ongoing intensive regeneration. Phytoplankton growth was very probably limited by light, as indicated by the Secchi depth which varied from 2 m at station SJ108 to 5 m at station RV001. Moreover, maximum chlorophyll *a* concentrations were determined in the top 5 m of the water column, except for station SJ108 where the maximum was deeper (5–10 m). Lack of high chlorophyll concentration in the surface layer of that station can probably be explained by the comparatively lower salinity (21.89), which was under the optimum for the growth of coastal phytoplankton.[25]

Since fucoxanthin strongly prevailed over all other biomarker pigments and fucoxanthin/chlorophyll *a* ratio of 1.5 was close to the literature value for diatoms,[26] we concluded that diatoms were responsible for the bloom that occurred in the brackish layer. This conclusion is in very good agreement with the microscopic examination of the samples, which showed a strong predominance of two diatom species, *Chaetoceros socialis* and *Chaetoceros sp.*[16] The abundance of these species reached a maximum in the middle part of the basin (19×10^6 cells/l), which is well matched by the distributions of fucoxanthin and chlorophyll *a* (Figure 3). The phytoplankton population in the bottom layer was dominated by the same two diatom species, but their counts were significantly lower ($< 10^5$ cells/l), as reflected in a very similar chlorophyll-to-fucoxanthin ratio (1.4). The nanoplanktonic flagellates present in the bottom layer at stations SJ108 and SJ101 could not be identified by conventional inverted microscopy. The biomarker pigment assemblage dominated by 19′-butanoyloxyfucoxanthin at these stations suggested that they belonged to chrysophytes (silicoflagellates)[21] induced by the increased availability of regenerated orthosilicate.

The diatom bloom in the brackish layer was associated with a pronounced increase of total carbohydrates, while those in the underlying layers were markedly lower. In fact, the concentrations in the seawater layer were rather similar to those found in open ocean waters[1] using the same analytical method. The concentration of total carbohydrates in the Atlantic and Pacific oceans[1] varied from 84 to 396 μg C/l, which is very similar to the concentration range in the seawater layer in this work (69–234 μg C/l). On the other hand, the total carbohydrate concentration in the brackish layer was comparable to those reported for estuarine waters.[3] Senior and Chevolot[3] reported concentrations of total carbohydrates mostly varying in a very wide range from 20 to 570 μg C/l, but high values up to 1080 μg C/l were also noted in the period of increased biological activity during summer. Enhanced carbohydrate levels were attributed to phytoplankton production but no information was provided which would allow their linking to the taxonomic composition of phytoplankton. Ittekkot *et al.*[5,6] indicated that the en-

hanced levels of carbohydrates were associated with their release during the senescent phase of a diatom bloom and suggested that this was an important source of dissolved carbohydrates in the marine environment. Our data confirm this hypothesis and show a statistically significant correlation between the fucoxanthin-containing biomass and total carbohydrates ($r^2 = 0.59$, $n = 31$, $p < 0.001$), indicating a large potential of *Cheatoceros* family to produce and accumulate carbohydrates. This potential was previously well-documented in laboratory experiments.[27,28]

Since the samples analyzed during this study were not filtered, we have no direct evidence of the carbohydrate percentage in the particulate and/or dissolved forms, *i.e.* how much of the produced carbohydrates was released out of the cells. An estimate assuming an average C/chlorophyll ratio of 50[29] leads to the conclusion that up to 1000 μg/l of carbohydrates in the brackish layer must have been in the dissolved form. This would indicate a strong release of carbohydrates by *Chaetoceros* cells.

Despite the significant correlation of total carbohydrates with chlorophyll *a*, which revealed diatoms as the main source, a closer comparison of the spatial distribution of chlorophyll *a* (or fucoxanthin) and total carbohydrates indicated some important differences. A high concentration of chlorophyll *a* was recorded at all stations along the transect SJ101–SJ107, while the largest increase of total carbohydrates occurred only at stations SJ105 to SJ107. It suggested that not only a high diatom crop but also its physiological status must have contributed to the observed distribution. Similarities of the spatial distribution of total carbohydrates and of breakdown products of chlorophyll *a* (Figure 5) in the brackish layer indicated that the production of carbohydrates was associated with increasing senescence of the bloom. The highest concentrations of carbohydrates were found in the samples having the highest concentration of chlorophyllide *a*. This breakdown product is formed by the cleaving off of phytyl chain from the chlorophyll molecule by the enzyme chlorophyllase, which was shown to be rather abundant in diatoms.[30] Linking of the enhanced total carbohydrate concentrations to the senescence of the diatom bloom is in agreement with previous reports by Ittekkot *et al*.[5,6] Senior and Chevolot[3] also determined a significant correlation of carbohydrate concentrations with phaeopigments determined by the conventional fluorimetric method.[31] Moreover, our data suggest that the other two diagnostic phaeopigments, phaeophorbide a_1 and phaeophorbide a_2, were also more abundant in the middle part of the basin. Since phaeophorbide a_2 was recommended as a specific biomarker of grazing,[32] some carbohydrate release could have been caused by sloppy feeding of herbivorous grazers. Indeed, a significant number of potential grazers were found in autumn 1993 in the central northern Adriatic.[33]

The senescence of the diatom bloom was most probably caused by progressive depletion of nutrients, notably orthosilicate and nitrate, which fell

below the level of 2 μmol/l. Exhaustion of nitrate was suggested as one of the main reasons for the channelling primary production into storage carbohydrates.[34] Such observations are in agreement with numerous laboratory experiments, which showed that accumulation and subsequent release of carbohydrates started when most of the present nitrogen was used up.[9,27,28] It was also shown that the production of carbohydrates strongly depended on the N/P ratio in the medium, with a much higher release of carbohydrates under P-limited conditions.[35]

A large percentage of the carbohydrates determined in the northern Adriatic was in combined form. This is in agreement with previous laboratory[27,28] and field studies[1,3–6] which suggest that phytoplankton excrete primarily storage glucans. Monosaccharides were a minor fraction of total carbohydrates and showed a different spatial distribution along the transect SJ101–RV001, with gradually increasing concentration towards the eastern side of the basin. They were very probably formed by *in situ* hydrolysis or partial microbial degradation of polysaccharides, as suggested by Ittekkot *et al.*[6]

The observed levels of carbohydrates in the northern Adriatic are high and could be of a great ecological significance. Easily biodegradable storage glucans may lead to an intensive oxygen consumption in the bottom layer, causing a strong bottom anoxia or hypoxia after the collapse and sedimentation of a diatom bloom. Furthermore, the role of carbohydrates in the formation of mucous aggregates has to be mentioned. The importance of carbohydrates as the main chemical constituents of mucilage was recognized by many workers (see Degobbis *et al.*[8] for review and references) but the mechanism leading to the phenomenon is still to be elucidated. No mucilage was observed during the event in October 1993 but our measurements certainly showed that phytoplankton species that occur in the northen Adriatic have a large potential for carbohydrate production. To our best knowledge, this is the first paper that reports on enhanced carbohydrate levels in the northern Adriatic and documents a possible link to diatoms as a major source.

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SAŽETAK

Raspodjela ugljikohidrata tijekom dijatomejskog cvata u sjevernom Jadranu

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Proučena je raspodjela ugljikohidrata u sjevernom Jadranu tijekom jesenskog cvata fitoplanktona koji je bio potaknut intenzivnim donosima rijeke Po na kraju razdoblja stratifikacije u listopadu 1993. Ukupni ugljikohidrati i monosaharidi određeni su spektrofotometrijski upotrebom metode MBTH, dok je dinamika planktona praćena određivanjem klorofila i karotenoida metodom HPLC. Istovremeni snažni porast klorofila *a* i fukoksantina pokazao je da se u površinskom sloju (< 5 m) sjevernog Jadrana razvio intenzivan dijatomejski cvat, dok je koncentracija biomase u dubljim slojevima (>10 m) bila vrlo niska. Koncentracija ukupnih ugljikohidrata varirala je u širokom rasponu od 70 μg C/l do 1300 μg C/l s izrazito povišenim vrijednostima u površinskom sloju, sugerirajući jaku povezanost s dijatomejskom biomasom. Međutim, prostorna raspodjela ugljikohidrata pokazala je da povišenje njihove koncentracije nije bilo povezano samo s koncentracijom nego i s fiziološkim stanjem (senescencijom) biomase, koje se odrazilo u sastavu razgradnih proizvoda klorofila *a*. Najzastupljeniji oblik ugljikohidrata bili su polisaharidi, predstavljajući do 92% njihove ukupne koncentracije.